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A SIEVE DEVICE FOR SAMPLING AIR-BORNE MICROORGANISMS¹

By H. G. DUBUY, *Associate Physiologist*, and L. R. CRISP, *Associate Mechanical Engineer, United States Public Health Service*

A sieve device for the sampling of air-borne microorganisms has been constructed. As in most other impinging devices, use is made of the standard petri dish. Its advantages over the other impinging devices are that it is compact and the inlets cover an area approaching that of the open plate. Thus, not only the floating nonsettling microorganisms are impinged, but also those which settle over an area normally covered by an open plate.

For obtaining samples of air-borne bacteria, the sieve device uses the impinging principle as employed first by Winslow (1) in 1908, who drew air into two flasks with a layer of nutrient agar in the bottom, on which microorganisms contained in the air were impinged. Hollaender and Dalla Valle (2) impinged the air on a standard petri dish, using a funnel in place of the bottle opening to impinge the air on the agar surface. In the sieve device, the air current is directed toward the agar surface by small openings in a sieve plate kept at a short distance from the agar surface. The bottle device of Sharf (3) furnished some indication that the use of small openings might not materially affect the sampling efficiency, since in that device the air is allowed to enter through a relatively small opening. The slit device as developed by Bourdillon et al. (4) furnished a method of determining the distance of the impinging inlet to the agar surface. The description of an additional sampling device of the impinger type might be justified, since in our hands the sampling efficiency of this device compares favorably with that of other impinger devices tested.

The device consists of two parts, a box which is equipped with an air outlet and holds a standard petri dish with nutrient agar, and a cover consisting of a brass plate with 300 openings each 0.796 mm. in diameter (No. 68 drill). This cover fits the box airtight and is fastened by means of two toggle clamps. Figure 1 shows a model in which a bayonet type lock is used. However, the use of toggle clamps simplifies the exchange of plates.

¹ From the Industrial Hygiene Research Laboratory, National Institute of Health.

The sieve plate itself can be adjusted to any desired distance from the agar surface of the petri dish, by movement along the screw thread of the plate and the margin of the cover, by means of wing handles on the sieve plate. The distance between the sieve plate and the agar surface is given by an indicator in the middle of the plate. In case a bayonet type lock is used, the indicator should be provided with a clevis to prevent it from making a hole in the agar while adjusting the sieve plate. Details for construction and essential dimensions are given in figure 2. The latest model is made of plastic except for the sieve plate and a small ring carrying the screw threads. These parts are made of brass.

The air enters through the openings of the sieve plate and impinges on the agar surface. It then passes around the petri dish to the center outlet on the bottom of the box and the outlet tube to a flow-meter and a suction pump. The air volume passing through the openings in the sieve plate is determined by the inside diameter of the outlet tubing, since the total surface of the 300 openings is larger than that of the outlet tubing. In our case, the diameter of this outlet is 4 mm., allowing sampling at varying rates of airflow. The bottom of the box is provided with four spacer pins of 0.7 mm. thickness, which support the petri dish and allow the air to pass to the outlet. In case samples have to be taken through small openings from an experimental room, three or four funnel clamps can be attached to the margin of the cover of the sieve device, while a washer prevents air leakage between funnel and cover. (See fig. 2, funnel clamp.) The addition of a funnel cuts the efficiency of the sieve device down to that of the funnel device, or roughly 20 percent. Before use the sieve plate is wiped with ethanol and flamed.

TABLE 1.—*Bacterial counts obtained by exposing open plates for 10 minutes and by taking 10-minute air samples with the funnel and sieve samples at a flow rate of 1 cu. ft. per minute. In the case of the open plates each run is represented by the average of six plates. Room supplied with dust*

Method	Number of runs	Bacterial count per 10 cu. ft. of air	
Open plate.....	27	18.8	±4
		Exposed 10 min.	
Funnel device.....	27	70.4	±7.5
Sieve device, 150 holes.....	27	82.4	±6.9

In order to determine the sampling efficiency of the sieve device, air samples were taken simultaneously with the funnel device, which in our hands was the least variable of the already existing impinger devices. (Atomizing devices, by breaking up particles, have a much higher "efficiency.") The counts obtained are represented in table 1. Each run consists of the exposure of one petri dish in the funnel

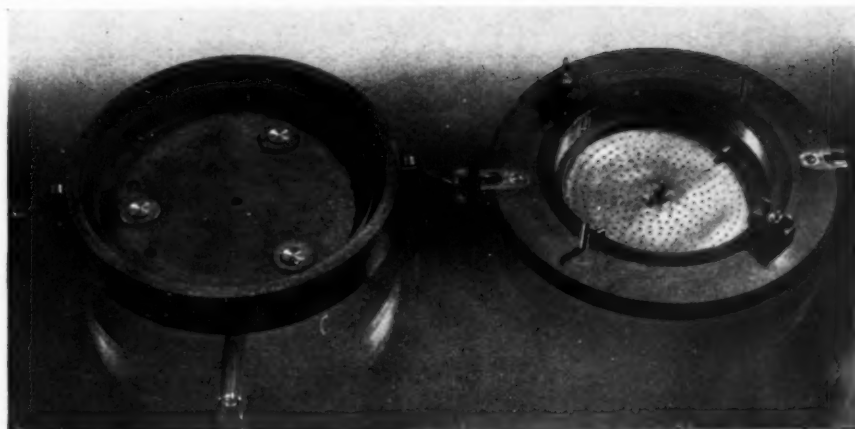


FIGURE 1.—Photograph of box and cover of the sieve sampling device. Notice toggle clamps and funnel holders. The holes in the sieveplate have been made at equal distance from each other in order to secure proper spacing of the bacterial colonies.

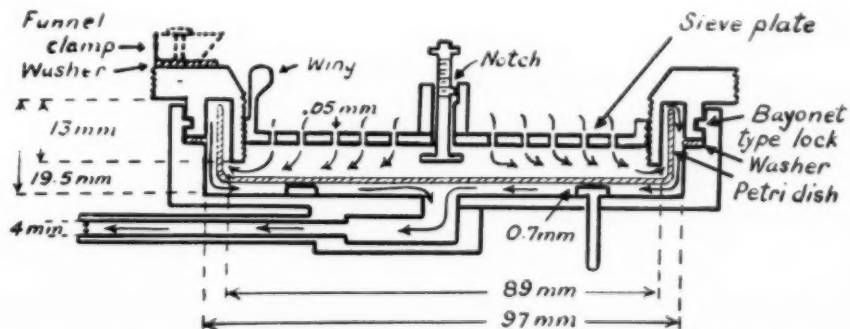


FIGURE 2.—Cross section through both parts of the sieve sampling device. The air path is indicated by arrows. In case toggle clamps are used to lock box and cover, the clevis and notch of the indicator are no longer necessary. (See fig. 1.)

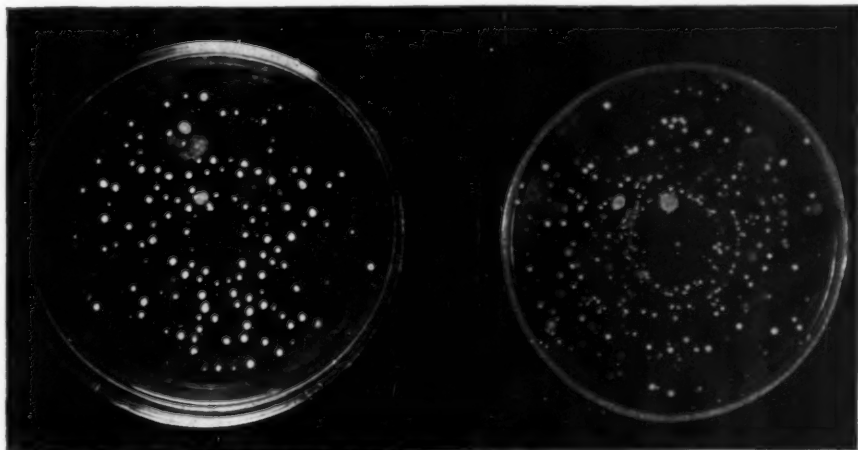
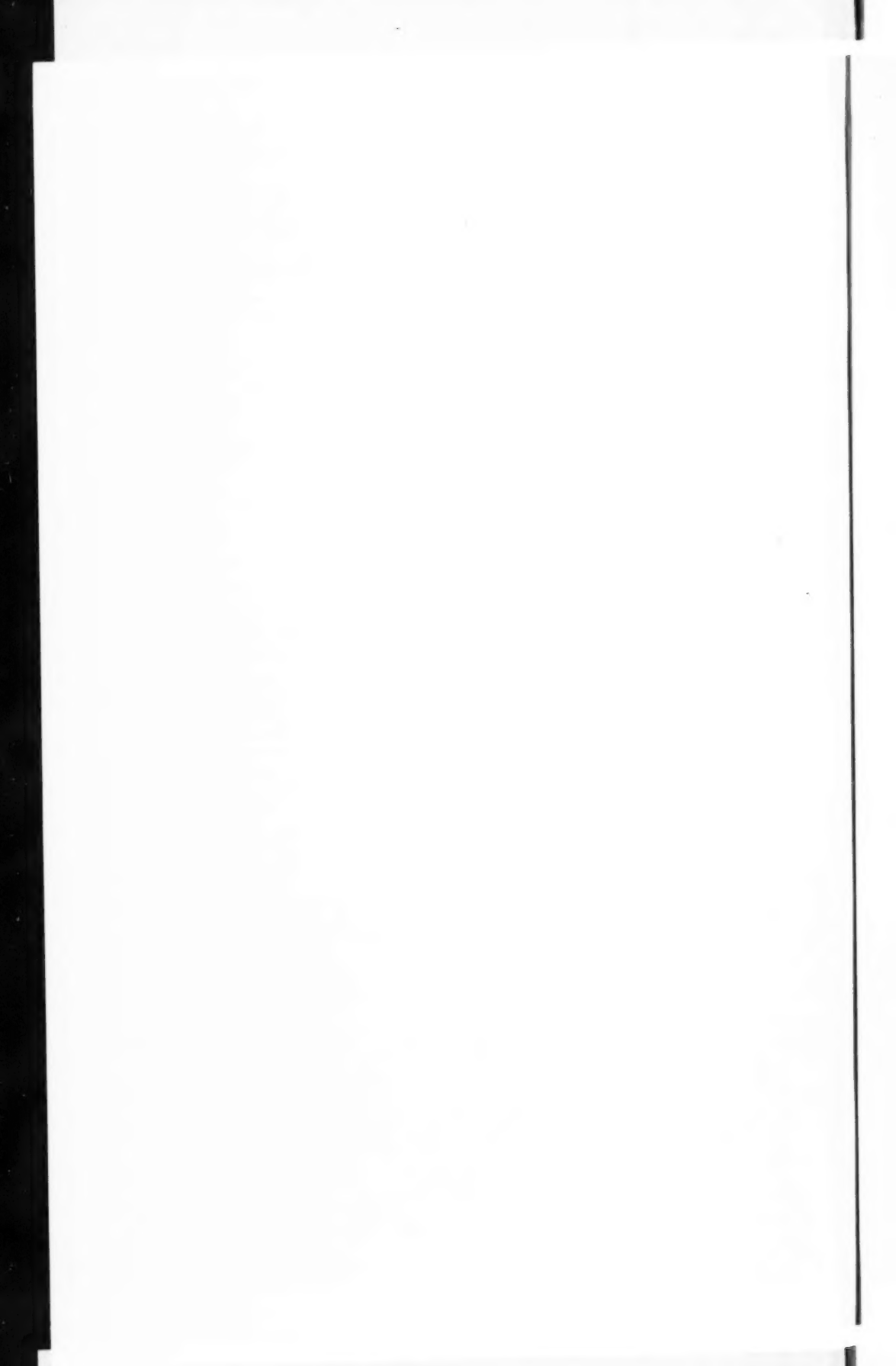


FIGURE 3.—Distribution of colonies over an agar plate used in the sieve sampler. Both plates represent samples of 10 cu. ft. at the rate of 1 cu. ft. per minute. (A) Sample of relatively pure air. (B) Sample of heavily contaminated air. Samples of more than 500 organisms per plate make counts less reliable as a result of overlapping.



device and one in the sieve device, simultaneously with the exposure of six open plates placed at strategic locations around the experimental room. The results show an increase in bacterial counts with the sieve device as compared with the funnel device, when a sieve plate with 150 openings is employed. The increase becomes more pronounced when a sieve plate with 300 openings is used. This increase runs parallel with a less pronounced increase of the values for the standard deviation.

TABLE 2.—*Bacterial counts obtained from room sprayed with E. coli, runs made as in table 1. Relative humidity of 35–45 percent*

Method	Number of runs	Bacterial count per 10 cu. ft. of air	
Open plate.....	18	7.7	±2.5
		Exposed 10 min.	
Funnel device.....	18	40.6	±5.4
Sieve device, 150 holes.....	18	50.2	±6.4
Sieve device, 300 holes.....	18	77.7	±7.9

This increase is partially due to the fact that the funnel device, like all other sampling devices, forces the air through one limited opening, thus preventing the impingement of those organisms, which slowly settle some distance away from this opening. In the case of the sieve device, practically all organisms settling over the area of a petri dish will enter through one of the many openings of the sieve plate. After passage through the sieve plate they will spread somewhat, since each small opening gives rise to a miniature vortex. Figure 3 demonstrates the spread of the organisms when relatively pure air (A) or heavily contaminated air (B) is sampled. It is possible that two or more organisms, floating separately in the air, will hit the same place on the agar surface, but this chance is very small since they will enter through any one of the 300 holes and they will have various angular velocities while passing through the openings and the vortices underneath each opening, and thus only occasionally hit the same spot. The data show that the sampling efficiency does not decrease appreciably when the air velocity decreases. On the other hand, with velocities at the rate of 40 liters per minute or higher, decreases in total bacterial count are found due to a relative decrease in the number of marginal colonies. This result is even more pronounced when the diameter of the openings in the sieve plate is gradually decreased toward the margin. This was done in one case in order to counterbalance the effect of the decrease of resistance to airflow toward the margin of the sieve plate, which is caused by the gradually decreasing distance between inlet openings and outlet, indicating that in the sieve sampler complex relations exist between air velocity and direction of airflow. On the basis of experimental

results, all openings in the sieve plate have been made of the same diameter in the latest model.

TABLE 3.—*Relation between bacterial counts per air sample of 10 cu. ft. and rate of airflow through sieve device. Sieve plates with 150 openings. Room supplied with dust. Three sieve samplers were employed simultaneously*

Cu. ft./min.	Number of runs	Number bact./10 cu. ft.	Cu. ft./min.	Number of runs	Number bact./10 cu. ft.
0.25.....	6	27 ±6.4	1.5.....	6	20 ±4.4
.5.....	14	31 ±5.2	1.9.....	6	16 ±.7
1.0.....	19	30 ±3.7			

The data presented here as well as others on the comparison of this device with various sampling devices, which will be published elsewhere, show that the bacterial counts per unit of air volume of the sieve device compare favorably with those of other impinging devices.

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PRODUCTION OF VITAMIN K DEFICIENCY IN RATS BY VARIOUS SULFONAMIDES ¹

By A. KORNBERG, *Passed Assistant Surgeon (R)*, F. S. DAFT, *Principal Biochemist*, and W. H. SEBRELL, *Medical Director, United States Public Health Service* ²

Vitamin K deficiency in rats is manifested by hypoprothrombinemia and hemorrhages. Feeding of a vitamin K-free ration results in an irregular production of this deficiency (1, 2). The inclusion of sulfonamides in purified diets is more uniformly effective. The occurrence of hemorrhages in rats ingesting such diets was reported by Daft, Ashburn, and Sebrell (3). Black et al. (4) found that the inclusion of 0.5 percent of sulfaguanidine or succinyl sulfathiazole in diets of rats for 4 weeks resulted in a significant increase in the prothrombin time of diluted plasma which could be prevented by vitamin K. However, Black et al. were unable to demonstrate a prolongation

¹ From the Division of Physiology, National Institute of Health.

² With the technical assistance of Howard Bakerman.

of the clotting time of whole blood and did not report the presence of hemorrhages. Welch and Wright (5) noted "spontaneous bleeding" occasionally, and hypoprothrombinemia in rats fed on a 2 percent succinyl sulfathiazole diet over a period of several weeks. In the present studies, it is established that the hemorrhages originally reported (3) were in all probability the result of a deficiency of vitamin K. Other sulfonamides (sulfapyrazine,³ sulfadiazine, and sulfathiazole) have been used which have been found to produce a severe hypoprothrombinemia and widespread hemorrhages rapidly and consistently. Further data are reported concerning the production, prevention, and correction of this vitamin K deficiency.

METHODS

Albino rats of Wistar or Osborne and Mendel strains, upon weaning at about 22 days, were given an experimental diet or a control diet. The experimental diet was composed of glucose ("Cerelese") 72 percent, casein 18 percent, cod liver oil 2 percent, cottonseed (Wesson) oil 3 percent, salt mixture No. 550 (6) 4 percent, and one of the sulfonamide drugs at a level of 1 percent. Each rat received a daily supplement of 100 micrograms of thiamine hydrochloride, 200 micrograms of riboflavin, 100 micrograms of pyridoxine hydrochloride, 200 micrograms of calcium pantothenate, 1 mg. of niacin, and 10 mg. of choline chloride. The control diet was identical except that the sulfonamide drug was replaced by an equal weight of glucose.

Casein, leached and alcohol-extracted in this laboratory, and Smaco ("vitamin test") casein were used for the most part. Other caseins (Labco, crude and leached) were also used in an experiment for the study of differences between various types of casein (table 3).

In all experiments litter mates were of the same sex and comparable weights. The rats were housed in individual metabolism cages to discourage coprophagy and permit the collection of feces.

A complete autopsy was performed on experimental and control rats. Microscopic examination of the tissues of some of the animals was carried out as described elsewhere (7).

Prothrombin time was determined by a micromethod adapted from a test described by Ziffren et al. (9).⁴ Thromboplastin (4 cu. mm. beef lung, Abbott) is delivered on a glass slide. From the first drop of tail blood, 15 cu. mm. is removed and added to the thromboplastin. The mixture is stirred with a fine glass rod. The clot normally occurs about 30 seconds after contact of blood with thromboplastin. This time may vary from 20 to 40 seconds for different samples of thromboplastin. For a given sample, freshly prepared, the prothrombin time is relatively constant within any control group. Determinations on control and experimental rats were made at the same time and with the same thromboplastin. In control rats the clot was complete, firm, and elastic. In rats with marked hypoprothrombinemia, the clot was delayed, incomplete, and friable. In such cases, the first evidence of a fibrin strand was considered to be the end point. Observations were not carried beyond 600 seconds.

It is recognized that prothrombin time is not necessarily a measure of the level of prothrombin in the blood. Such terms as "blood clotting power," "prothrom-

³ Furnished through the courtesy of Dr. Warren Cox, Mead Johnson & Co.

⁴ Suggested in a personal communication by Dr. H. P. Smith, University of Iowa, Iowa City, Iowa.

bin level," and "prothrombin activity" have been used to indicate the possible blood prothrombin level as measured by prothrombin time. In this paper we have used the term "prothrombin level" in this sense. A numerical indication of blood prothrombin level is expressed as a percent. The average prothrombin time of a group of control rats (or a single, litter mate, control rat) is divided by the prothrombin time of the experimental rat and multiplied by 100.

The term "hypoprothrombinemia" was applied only to those rats whose prothrombin levels had fallen below 30 percent. Although some depletion of prothrombin was probably present at higher percentages, i. e., 30 to 50 percent, the designation of hypoprothrombinemia has been rigidly reserved for levels under 30 percent.

Hypoprothrombinemic rats were used for treatment with pure vitamin K⁵ and for assay of the vitamin K activity of crude substances. In regard to their responses, it appeared to make no difference whether the hypoprothrombinemia was induced by one sulfonamide or another. In the assay of crude materials, the substance in question was administered orally within a few hours after a prothrombin determination had been made. Eighteen to 24 hours later, the prothrombin determination was repeated. The rats always continued to ingest the sulfonamide-containing diet during the treatment or assay period.

The whole blood-clotting time was determined on the first drop of tail blood collected in a capillary tube. Pieces (1 cm.) of the tube were broken at 15-second intervals and when a fibrin strand was seen to connect the broken ends of the tube, clotting was considered to have occurred. Values from rats on control diets were within the range of 60 to 120 seconds.

In the experiment designed to study the production of a vitamin K deficiency by sulfadiazine and sulfathiazole and its prevention (table 1, fig. 1), groups of 3 litter mates were used. Two rats from each litter were placed on the experimental diet containing the sulfonamide. One of these 2 rats was given orally by pipette 40 micrograms of 2-methyl-1,4-naphthohydroquinone diacetate three times weekly. The third litter mate received the control diet. Smaco casein was used. Determinations of prothrombin time were made weekly for 10 weeks. The prothrombin time of the control rat served as the standard for its litter mates. Upon the death of 1 rat the 2 litter mates were sacrificed.

The experiment in which various sulfonamides were compared as to effectiveness in producing vitamin K deficiency (table 2) was set up with groups of 4, 5, 6, and 7 litter mates. One member of each litter was fed the control diet and the others were fed the various sulfonamide experimental diets. Smaco casein was used. Prothrombin determinations were made weekly for 10 weeks.

Investigation of the effect of biotin and "folic acid" (*L. casei* factor)⁶ on the production by sulfadiazine of vitamin K deficiency (table 4) was conducted with groups of 4 litter mates. The experimental diet containing sulfadiazine was used. One rat was given crystalline biotin, one crystalline folic acid, one was given both of these vitamins, and the remaining litter mate was given neither. The biotin (5 micrograms) and folic acid (5 micrograms) were given orally by pipette each day. Smaco casein was used. Determinations of prothrombin time were made at 2, 3, and 4 weeks after the start of the experiment.

⁵ The vitamin K preparation used throughout these studies was 2-methyl-1,4-naphthohydroquinone diacetate. The potency was found to be one-half that of 2-methyl-1,4-naphthohydroquinone (Menadione) as determined by chick assay (10).

⁶ The crystalline material used in the present studies was furnished through the courtesy of Lederle Laboratories. The source was not given but it was stated not to be identical with either of the substances described by Stokstad (11) as "a growth factor for *Lactobacillus casei*." The potency of the material was as follows: 0.000061 micrograms per cc. gave half maximum growth of *L. casei* and 0.0042 micrograms gave half maximum growth of *Streptococcus lactis* R.

RESULTS

Production of vitamin K deficiency by sulfadiazine or sulfathiazole and its prevention.—Feeding experimental diets containing sulfadiazine or sulfathiazole was found to produce a severe hypoprothrombinemia, prolonged clotting time, and multiple hemorrhages. These abnormalities were preventable by orally administered vitamin K (table 1, fig. 1). It may be noted that on the experimental diet prothrombin levels under 30 percent were recorded in 18 of 21 rats. Rats getting the experimental diet and in addition regular doses of vitamin K maintained prothrombin levels at or near those of their litter mates on the control diet (fig. 1). In some of the rats getting vitamin K supplements, somewhat low prothrombin levels were noted on

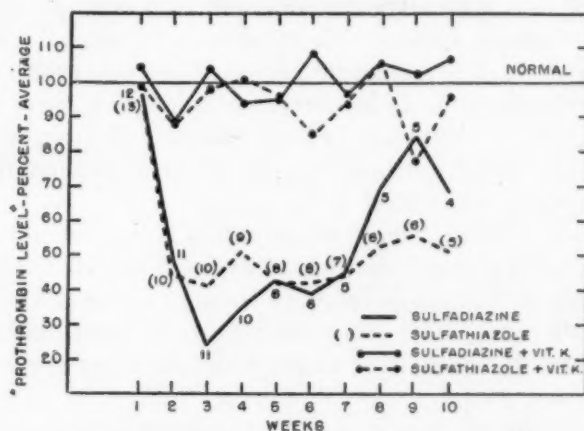


FIGURE 1.—The course of hypoprothrombinemia produced by sulfadiazine and sulfathiazole and prevented by vitamin K. (The numbers along the sulfadiazine and sulfathiazole lines indicate the number of rats whose individual values are averaged at a particular week. The lines for sulfadiazine+vitamin K and sulfathiazole+vitamin K are made up from values of the same numbers of rats (litter mates) indicated for the sulfadiazine and sulfathiazole lines respectively.)

single occasions, but normal levels were found prior to and subsequent to these low determinations.

TABLE 1.—Hypoprothrombinemia produced by sulfadiazine and sulfathiazole and prevented by vitamin K

Drug	Number of rats	Lowest individual prothrombin levels ¹ (percent)	Average of lowest prothrombin levels (percent)
Sulfadiazine	11	5, 5, 7, 11, 16, 19, 22, 24, 24, 31, 33	18
Sulfadiazine+vitamin K ²	11	69, 70, 70, 77, 78, 82, 82, 88, 90, 92, 93	81
Sulfathiazole	10	6, 8, 14, 19, 19, 23, 25, 26, 29, 41	21
Sulfathiazole+vitamin K ²	10	40, 50, 64, 66, 75, 75, 76, 80, 86, 88	70

¹ Determinations were made weekly for 10 weeks. The lowest level reached by each rat is recorded here. Litter mates were used.

² 40 micrograms given orally by pipette 3 times weekly.

In following the weekly prothrombin levels of individual experimental rats, the lowest values were noted at the second and third week in 13 of the 21 rats. Some degree of remission occurred in all rats (except in 2 which died early of acute hemorrhage), which was generally slight and usually followed by relapses to previous levels. In 5 rats, there was a remission which over a period of weeks elevated the prothrombin level to near normal values.

Hemorrhages were noted in a variety of sites, the most common being the subcutaneous tissues of the lower extremities. Other sites where bleeding occurred with some frequency were the thymus, bladder, epididymis, eye, adrenal, testicle, stomach, kidney, retroperitoneal space, and the thoracic, abdominal, and cranial cavities. The hemorrhagic thymus had a striking appearance. The gland was purplish black in color and symmetrically enlarged to occupy as much as one-half of the thoracic cavity. Eight of the 11 animals which had prothrombin levels of less than 20 percent were observed at the time of the determination to have hemorrhages in one or more of the above-enumerated places. Evidences of spontaneous bleeding were rarely observed in rats with "prothrombin levels" over 30 percent and were never found in rats on control diets.

Whole blood clotting times of 10 rats whose prothrombin levels were less than 30 percent showed an average of 276 seconds (range 120 to 600 seconds) as compared with their 10 controls whose average was 75 seconds (range 60 to 105 seconds). In rats with milder degrees of hypoprothrombinemia, little or no prolongation of the whole blood clotting time was found.

Two rats in the vitamin K deficient group died owing to massive hemorrhage. Thirteen rats died during the experiment from causes other than vitamin K deficiency. Six of these received vitamin K supplements and 7 were vitamin K deficient. No differences in weight gain were observed between rats developing a vitamin K deficiency and litter mates in which it was prevented.

The production of a vitamin K deficiency was also studied in rats which had ingested the control diet for 3 weeks after weaning and were then given an experimental diet containing 1 percent sodium sulfadiazine. Litter mates of the same sex and starting weights were fed this experimental diet immediately upon weaning. The average weight at weaning was 35 gm. and after 3 weeks on the control diet it was 80 gm. Prothrombin times were determined 2 weeks after the feeding of the experimental diet was started. The rats given the experimental diet at weaning did not develop a more severe vitamin K deficiency than rats given the experimental diet when somewhat older and heavier. The rats given the experimental diet at weaning had prothrombin levels of 37, 31, 13, 4, and 5 percent. Their litter

mates given the control diet for 3 weeks prior to the experimental diet had prothrombin levels of 12, 7, 15, 7, and 18 percent respectively.

Comparative effectiveness of various sulfonamides in producing a vitamin K deficiency.—Various sulfonamides were compared with respect to the severity of the vitamin K deficiency they produced and the rapidity of its production. The data in table 2 are from a representative experiment in which litter mates were observed for a 10-week period. It may be noted that the compounds fall into two groups. Sulfapyrazine, sulfadiazine, and sulfathiazole were more effective than sulfanilamide, succinyl sulfathiazole, or sulfaguanidine.⁷

TABLE 2.—Comparative effectiveness of various sulfonamides in producing a vitamin K deficiency

Drug	Number of rats	Lowest individual prothrombin levels ¹ (percent)	Average of lowest prothrombin levels (percent)	Average of prothrombin levels after 2 weeks on experiment (percent)
Sulfapyrazine.....	6	4, 9, 12, 18, 29, 30.....	17	17
Sulfadiazine.....	10	5, 6, 6, 9, 10, 15, 20, 26, 28, 33.....	16	31
Sulfathiazole.....	9	6, 11, 11, 11, 17, 19, 22, 41, 42.....	20	53
Succinyl sulfathiazole.....	8	24, 40, 43, 43, 48, 61, 64, 77.....	50	78
Sulfanilamide.....	6	4, 12, 42, 77, 83, 85.....	51	78
Sulfaguanidine.....	9	21, 26, 30, 31, 40, 76, 80, 85, 100.....	54	90

¹ Determinations were made weekly for 10 weeks. The lowest level reached by each rat is recorded here. Litter mates were used.

Sulfapyrazine was the most potent compound studied. Of 25 rats on the sulfapyrazine-containing diet, including 19 from other experiments, 20 developed a severe hypoprothrombinemia with prothrombin levels under 20 percent. Multiple hemorrhages were observed in 18 of these 20 rats. The other 5 rats had prothrombin levels between 21 and 30 percent. These manifestations were noted after only 2 to 3 weeks on the experimental diet. Fifteen rats in the sulfapyrazine group were used in treatment experiments. Nine of the 10 untreated rats died early with massive hemorrhages and 1 rat survived with a return of its prothrombin level to normal.

Influence of the type of casein upon development of vitamin K deficiency.—It was noted during the course of these various studies that the type of the casein in the diet played a part in the rapidity of production of the vitamin K deficiency and its severity. This was investigated more carefully by comparing litter mates on sulfonamide-containing diets which differed only in regard to the type of casein (table 3). Sulfadiazine was used in one experiment and sulfaguanidine in another. The two experiments gave parallel results. Crude or

⁷ Ten rats were fed the experimental diet containing 1 percent sulfamerazine. Hemorrhages and severe hypoprothrombinemia were observed in four of the rats between the second and third week. Observations were not continued beyond that time.

leached casein appeared to delay the onset of vitamin K deficiency and reduce its severity. Our leached and alcohol-extracted casein was the best of the caseins tested for use in diets designed to produce vitamin K deficiency.

An attempt was made to explain the differences in the results obtained with the leached and the leached and alcohol-extracted casein. The vitamin K activity of the material extracted from leached casein by alcohol⁸ was determined by the assay procedure with hypoprothrombinemic rats described later in this report. It was found to contain about 0.05 micrograms of vitamin K (2-methyl-1,4-naphtho-hydroquinone diacetate) activity per gram of leached casein.

TABLE 3.—*Effect of the type of casein in the diet on the production of vitamin K deficiency*

Drug	Casein	Number of rats	Lowest individual prothrombin levels ¹ (percent)	Average of lowest prothrombin levels (percent)	Average of prothrombin levels after 2 weeks on experiment (percent)
Sulfadiazine	Leached and alcohol-extracted. ³	9	3, 5, 5, 6, 10, 11, 19, 19, 41	13	25
	Smaco	8	5, 6, 8, 8, 9, 16, 42, 47	18	38
	Crude	8	6, 16, 20, 30, 32, 36, 40, 43	28	68
Sulfaguanidine	Leached and alcohol-extracted. ³	4	12, 16, 27, 63	30	58
	Smaco	5	17, 21, 46, 68, 69	44	77
	Labco	5	21, 26, 45, 81, 90	53	86
	Leached ²	5	53, 55, 67, 90, 91	71	90

¹ Determinations were made weekly for 10 weeks in the sulfadiazine group and biweekly for 8 weeks in the sulfaguanidine group. Groups of 4 and 5 litter mates were used of which 1 rat ate a control diet. The prothrombin level of the control rat receiving Smaco casein and no sulfonamide drug was taken as 100 percent.

² Leached for a week in daily changes of acidulated water (8).

³ Alcohol extraction of casein was by the following procedure: 400 gms. of dried, ground, leached casein was shaken with 2,000 cc. of 60 percent ethyl alcohol by volume for 30 minutes. The casein was filtered off the next day and the process repeated. Finally, it was washed with 1,000 cc. of 60 percent alcohol, then with 500 cc. of 95 percent ethyl alcohol and dried in air. 600 gms. of this casein was boiled for 4 hours in 1,200 cc. of 95 percent ethyl alcohol and filtered. This boiling was repeated 3 times and the casein dried in air.

Lack of effect of crystalline biotin and crystalline folic acid on the production of vitamin K deficiency.—It has been reported (5) that the effect of succinyl sulfathiazole on the prothrombin time of rats can be counteracted by crystalline biotin and folic acid concentrates. This report prompted the trial of these vitamins in experiments in which sulfadiazine was used to produce a vitamin K deficiency. Crystalline biotin and crystalline folic acid, either alone or together, did not appear to produce a significant change in the development of vitamin K deficiency (table 4). It may be noted that the deficiency produced in this one experiment was not as severe as usual, possibly because of the particular batch of casein used.

⁸ The alcohol filtrates were concentrated under reduced pressure and partially dried in vacuo over CaCl₂.

TABLE 4.—*Effect of biotin and folic acid on production of sulfadiazine vitamin K deficiency*

Supplement (oral)	Number of rats	Lowest individual prothrombin levels ¹ (percent)	Average of lowest prothrombin levels (percent)
None	5	10, 28, 36, 48, 56	35
Crystalline biotin, 5 micrograms daily	4	19, 29, 53, 69	43
Crystalline folic acid, 5 micrograms daily	5	12, 16, 25, 39, 78	34
Crystalline biotin and crystalline folic acid, 5 micrograms each daily	5	10, 26, 31, 44, 46	31

¹ Determinations were at 2, 3, and 4 weeks after start of experiment. Litter mates were used.

Further evidence was obtained that folic acid was not a determining factor in the development of vitamin K deficiency. Granulocytopenia and anemia which are produced by these various sulfon-

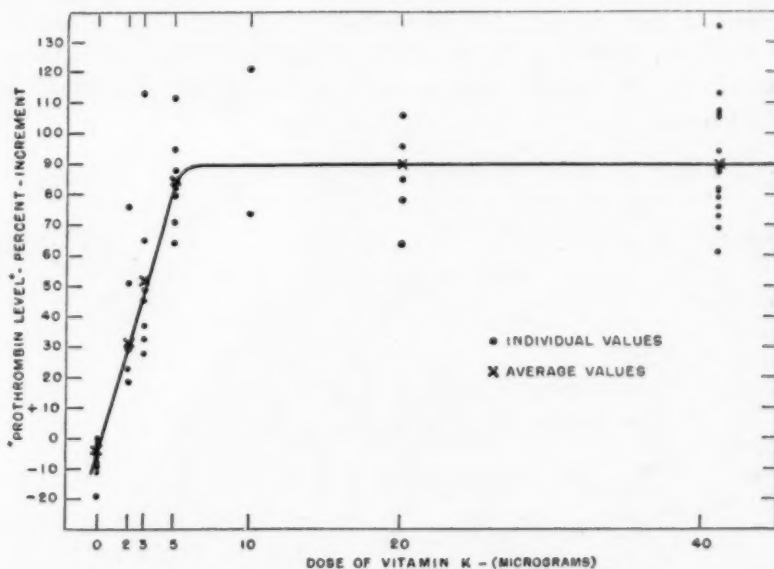


FIGURE 2.—Responses of hypoprothrombinemic rats to vitamin K (2-methyl-1,4-naphthohydroquinone diacetate).

amides (6, 12) are due to a lack of folic acid (13). Yet no correlation was observed between the development of these blood dyscrasias and vitamin K deficiency.

Treatment with vitamin K. Suggested vitamin K assay method.—Rats with hypoprothrombinemia induced by sulfapyrazine, sulfadiazine, and sulfathiazole gave uniformly rapid and consistent responses to the oral administration of 2-methyl-1,4-naphthohydroquinone diacetate (table 5, fig. 2). (The few rats with hypoprothrombinemia produced by sulfaguandine, sulfanilamide, and succinyl sulfathiazole were not treated.) The prothrombin level was found to attain a maximum by 10 hours after administration and

no further increase was observed between 10 and 24 hours. The responses were independent of the rapidity with which the hypoprothrombinemia was produced and the sulfonamide which produced it. Five micrograms was the least amount which regularly gave a complete response. Doses of 2 and 3 micrograms resulted in partial responses. Although spontaneous remissions have been noted, no evidence has been obtained to indicate that the recovery of untreated rats is ever abrupt. In general, when no treatment was given a repetition of the determination in 24 hours showed no change or a further decline in prothrombin level. A similar result was obtained when vitamin K-free substances were administered. A mixture of p-aminobenzoic acid (15 mg.), ascorbic acid (30 mg.), and crystalline biotin (30 micrograms) was given orally to 2 rats with prothrombin levels of 7 percent and 27 percent; the values were 5 percent and 22 percent, respectively, 24 hours later. A folic acid concentrate from liver was given to 3 rats with prothrombin levels of 6 percent, 7 percent, and 9 percent; the values were 5 percent for each of them after 24 hours.

TABLE 5.—Responses of hypoprothrombinemic rats to vitamin K¹

Amount of vitamin K given orally (micrograms)	Number of rats	Prothrombin levels before and after treatment (percent)	Average of prothrombin levels before and after treatment (percent)	Interval between treatment and final determination (hours)
40	15	Before treatment: 4, 5, 5, 5, 6, 6, 8, 9, 10, 11, 11, 11, 13, 19, 24. After treatment: 80, 94, 74, 78, 112, 111, 96, 103, 145, 124, 92, 118, 74, 101, 103.	10 100	24
20	5	Before treatment: 6, 6, 8, 10, 16. After treatment: 91, 112, 86, 74, 112.	9 95	12
10	2	Before treatment: 4, 12. After treatment: 125, 86.		17
5	8	Before treatment: 3, 5, 5, 6, 8, 9, 9, 11. After treatment: 67, 100, 117, 89, 92, 89, 80, 97.	7 91	10
3	7	Before treatment: 7, 8, 8, 9, 9, 16, 17. After treatment: 72, 45, 121, 54, 58, 49, 45.	11 63	10
2	5	Before treatment: 3, 5, 6, 20, 28. After treatment: 26, 36, 82, 71, 47.	12 44	24
10	11	Before treatment: 6, 8, 15, 18, 20, 22, 25, 25, 26, 26, 27. After treatment: 7, 8, 11, 6, 17, 13, 20, 19, 7, 18, 25.	20 14	24

¹ 2-methyl-1, 4-naphthohydroquinone diacetate.² No treatment given.

Following treatment with 5 to 40 micrograms and a full therapeutic response to vitamin K, 27 of the rats were maintained on the sulfonamide diet and prothrombin determinations were made at weekly intervals. When 4 weeks after original treatment had elapsed, 11 rats had developed a severe hypoprothrombinemia again, 3 retained

normal levels, 8 died, and 5 showed mild hypoprothrombinemia. The interval between treatment and relapse was as follows:

<i>Treatment dose micrograms vitamin K)</i>	<i>Interval between treatment and relapse (days)</i>
40.....	7, 19, 21, 21, 27, 27.
20.....	16.
10.....	7, 7.
5.....	7, 7.

Assay of vitamin K activity of crude substances by the use of rats made hypoprothrombinemic by sulfonamides is based on the specific and fairly uniform responses of such rats to pure vitamin K (table 5, fig. 2). The substance to be tested is administered orally to a rat whose prothrombin level is less than 30 percent. Determination of the prothrombin level is repeated 18 to 24 hours later. "Increases" over the pretreatment prothrombin levels of 60 percent or more, of 20 to 60 percent, and of less than 20 percent are considered to represent the following respective degrees of vitamin K (2-methyl-1, 4-naphthohydroquinone diacetate) activity: 5 micrograms or greater, 2 to 4 micrograms, and less than 2 micrograms. By testing the unknown substance at more than one level the accuracy of the results may be increased.

DISCUSSION

By the choice of the proper sulfonamide, it has been found possible to produce, rapidly and consistently, a vitamin K deficiency so severe that it could be demonstrated by relatively crude means. There was a marked prolongation of the clotting time of whole blood which occurs only in extreme hypoprothrombinemia. Multiple, massive hemorrhages were common. Severe degrees of vitamin K deficiency appeared in 2 to 3 weeks in over 80 percent of the animals which received sulfapyrazine, sulfadiazine, or sulfathiazole.

Relatively mild symptoms of vitamin K deficiency were produced by sulfaguanidine, sulfanilamide, and succinyl sulfathiazole comparable to those previously reported by other workers (4, 5). It appears, therefore, that sulfapyrazine, sulfadiazine, and sulfathiazole are considerably more effective than sulfaguanidine, sulfanilamide, or succinyl sulfathiazole. A consideration of the rapidity of production of vitamin K deficiency as well as its severity indicates further that sulfapyrazine is more effective than sulfadiazine or sulfathiazole. As shown in table 2, prothrombin levels averaging under 20 percent were produced by sulfapyrazine in 2 weeks while it was necessary to administer sulfadiazine and sulfathiazole for longer periods of time in order to obtain equally low average prothrombin levels.

Rats with sulfonamide-induced hypoprothrombinemia gave uniformly rapid and consistent responses to the oral administration of 2-methyl-1,4-naphthohydroquinone diacetate (table 5, fig. 2). It

appeared to make no difference, in respect to the response, which sulfonamide produced the hypoprothrombinemia. Five micrograms or more of the diacetate uniformly restored the prothrombin levels to normal values. Two or 3 micrograms gave partial responses. The responses to 3 micrograms averaged somewhat better than to 2. This is the basis of the assay method which is described under "Results."

Welch and Wright (5) reported that supplements of a "folic acid" concentrate and crystalline biotin antagonized the increased prothrombin time produced by succinyl sulfathiazole in purified diets. Black et al. (4) noted an antagonism by a liver factor of a sulfaguanidine-induced hypoprothrombinemia. In the present study, folic acid and biotin appear to have no effect on the sulfadiazine-induced vitamin K deficiency.

The type of casein in the diet was found to be an important factor in the production of vitamin K deficiency. This was shown to be related, to some extent at least, to the vitamin K content of the casein.

We have observed spontaneous remissions in hypoprothrombinemic rats. Alterations in intestinal vitamin K synthesis might account for some of these remissions.

The gross and microscopic lesions observed in the rats included in these various studies have been reported previously (7). Except for hemorrhages, no differences were noted between hypoprothrombinemic rats and experimental rats with normal prothrombin levels. The low incidence of liver lesions and their relative mildness make it appear doubtful that the hypoprothrombinemia produced in these rats by ingestion of sulfonamide diets is a result of such lesions.

Rats subjected to bile duct obstruction or given diets containing petrolagar have a defective alimentary absorption of vitamin K. This absorptive inadequacy is considered to be the basis for the vitamin K deficiency produced in these rats. It is of interest to compare observations made on such rats (14, 15) with our data on rats with sulfonamide-induced vitamin K deficiency. This comparison suggests that in rats ingesting sulfonamide-containing diets, vitamin K is efficiently absorbed and utilized and that the requirements for this vitamin have not been increased.

The minimal curative dose of vitamin K given orally to rats made hypoprothrombinemic by sulfonamides was probably no larger than that given parenterally to rats with vitamin K deficiency induced by bile duct obstruction (14, 15) or petrolagar diets (15). This makes it likely that in the sulfonamide rats there was no serious interference with absorption of vitamin K. Furthermore, the fact that the response to treatment was complete within 10 hours suggests the rapid as well as efficient utilization of the orally administered vitamin K in sulfonamide rats. Observations were made also of the time inter-

val between the correction of a vitamin K deficiency and a relapse to hypoprothrombinemic levels. This time period was approximately the same in rats on a sulfonamide regime as in rats with bile duct obstruction or in rats ingesting a petrolagar diet (15). This makes it doubtful that the requirements for vitamin K in sulfonamide rats are significantly increased.

The order of effectiveness of sulfonamides in producing a vitamin K deficiency as demonstrated in the present studies is very similar to the order of drug activity as reported by White (16) in relation to bacteriostasis of coliform organisms in the intestines of mice. The vitamin K synthesis by *B. coli* in vitro has been shown to exceed by far that of a number of other intestinal bacteria which were tested (17). Although these data are drawn from different sources, the parallelism between the effectiveness of these sulfonamides in producing a vitamin K deficiency and their bacteriostatic potency against a known synthesizer of vitamin K is striking.

SUMMARY

Sulfapyrazine, sulfadiazine, or sulfathiazole fed to rats at a 1-per-cent level in purified diets resulted in a regular production of severe hypoprothrombinemia and hemorrhage in 2 to 3 weeks. Sulfaguanidine, sulfanilamide, and succinyl sulfathiazole were much less effective.

Vitamin K, orally, prevented this hypoprothrombinemic and hemorrhagic state.

Crystalline biotin and crystalline folic acid, alone or combined, did not influence the production of vitamin K deficiency by sulfadiazine.

The type of dietary casein may be important in the development of vitamin K deficiency. Alcoholic extracts of leached casein showed vitamin K activity.

Rats made severely hypoprothrombinemic with a sulfonamide gave uniform and consistent responses to orally administered vitamin K.

A method for assay of the vitamin K activity of crude substances is suggested.

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DEATHS DURING WEEK ENDED JUNE 17, 1944

[From the Weekly Mortality Index, issued by the Bureau of the Census, Department of Commerce]

	Week ended June 17, 1944	Correspond- ing week, 1943
Data for 93 large cities of the United States:		
Total deaths.....	8,290	8,483
Average for 3 prior years.....	8,049	
Total deaths, first 24 weeks of year.....	230,412	235,373
Deaths under 1 year of age.....	646	595
Average for 3 prior years.....	563	
Deaths under 1 year of age, first 24 weeks of year.....	15,042	16,362
Data from industrial insurance companies:		
Policies in force.....	66,618,073	65,545,543
Number of death claims.....	12,459	12,646
Death claims per 1,000 policies in force, annual rate.....	9.8	10.1
Death claims per 1,000 policies, first 24 weeks of year, annual rate.....	10.6	10.4

PREVALENCE OF DISEASE

No health department, State or local, can effectively prevent or control disease without knowledge of when, where, and under what conditions cases are occurring

UNITED STATES

REPORTS FROM STATES FOR WEEK ENDED JUNE 24, 1944

Summary

A total of 126 cases of poliomyelitis was reported during the current week, as compared with 71 last week and 136 for the corresponding week last year. Of the current cases, 59 occurred in North Carolina and Kentucky (42 and 17, respectively). Of the 136 cases reported for the same week last year, 58 cases occurred in California and 39 in Texas, or 70 percent in these two States. The cumulative total to date this year is 822, as compared with 894 for the same period last year and a 5-year median of 697 for the period.

Of the other 8 diseases for which comparative figures are available for the preceding 5 years, the incidence of only meningococcus meningitis and scarlet fever is above the respective 5-year median.

A new low has been recorded for smallpox. A total of 263 cases has been reported to date this year, as compared with 568 for the same period last year, and 554 in 1942, which is the lowest figure previously reported for the corresponding period. Only 4 cases were reported during the current week—3 in Wisconsin and 1 in Texas.

Although the incidence of typhoid fever to date is about 20 percent above that for the same period last year, it is slightly below the median of the past five years. The cumulative total this year to date is 2,004 cases, as compared with 1,666 for corresponding period last year.

Of 97 cases of endemic typhus fever reported during the current week, 37 cases occurred in Texas, 20 in Georgia, and 16 in Alabama. The total to date is 1,295 cases, as compared with 1,204 last year.

Since January, the mortality in 93 large cities has been slightly above the 3-year (1941-43) average for most of the weeks. For the current week, however, the figure is slightly below the 3-year average—8,556 and 8,601, respectively. The cumulative total to date is 238,969, as compared with 244,474 for the same period last year.

Telegraphic morbidity reports from State health officers for the week ended 1944, and comparison with corresponding week of 1943 and 5-year median

In these tables a zero indicates a definite report, while leaders imply that, although none was reported, cases may have occurred.

Division and State	Diphtheria			Influenza			Measles			Meningitis, men- ingococcus		
	Week ended		Med- ian 1939- 43	Week ended		Med- ian 1939- 43	Week ended		Med- ian 1939- 43	Week ended		Med- ian 1939- 43
	June 24, 1944	June 26, 1943		June 24, 1944	June 26, 1943		June 24, 1944	June 26, 1943		June 24, 1944	June 26, 1943	
NEW ENGLAND												
Maine	0	0	0				60	125	125	0	7	0
New Hampshire	1	1	0				17	7	7	0	2	0
Vermont	0	0	0				19	154	131	0	0	0
Massachusetts	1	3	3				548	1,009	759	9	18	4
Rhode Island	0	0	1				5	97	97	1	5	0
Connecticut	1	3	1		1	1	138	200	227	1	7	0
MIDDLE ATLANTIC												
New York	14	15	13	(1)	4	4	638	2,548	1,146	27	45	6
New Jersey	2	4	4		4	3	432	1,452	933	11	16	1
Pennsylvania	8	12	9		1		244	553	463	15	21	6
EAST NORTH CENTRAL												
Ohio	2	5	5		1	8	93	357	182	15	12	1
Indiana	0	4	3		2	1	30	146	63	3	9	1
Illinois	7	7	17		14	14	134	926	217	17	19	1
Michigan	9	9	4		4	1	345	1,611	508	10	23	0
Wisconsin	1	0	0		8	9	12	823	1,665	954	4	6
WEST NORTH CENTRAL												
Minnesota	9	2	1				117	272	91	2	2	1
Iowa	0	0	1				81	85	126	0	0	0
Missouri	2	2	1				39	99	65	14	17	0
North Dakota	1	1	1		36	4	13	68	11	0	1	0
South Dakota	3	0	0				5	50	7	0	0	0
Nebraska	2	1	1				39	97	52	0	1	0
Kansas	0	1	3		2	4	1	63	126	126	2	2
SOUTH ATLANTIC												
Delaware	0	0	0				1	19	9	0	1	0
Maryland	5	3	3		4	1	2	74	155	79	1	4
District of Columbia	5	0	0					46	60	60	3	2
Virginia	3	1	5		66	18	18	115	112	138	9	10
West Virginia	1	1	3		3	3		68	23	23	0	0
North Carolina	10	4	4			8		184	76	120	7	13
South Carolina	7	1	1		62	80	108	80	40	29	3	2
Georgia	2	2	4		3	12	13	29	26	42	2	3
Florida	2	0	2		6	6	6	64	33	45	7	5
EAST SOUTH CENTRAL												
Kentucky	2	3	3		1	2	2	16	42	42	3	3
Tennessee	2	5	3		8	6	10	21	66	50	3	4
Alabama	2	3	3		15	15	12	48	78	72	9	11
Mississippi	1	2	1								4	0
WEST SOUTH CENTRAL												
Arkansas	3	3	3		8	1	8	63	22	22	1	4
Louisiana	2	3	3		1	1	5	42	13	13	7	1
Oklahoma	1	0	1		4	16	7	82	67	60	1	0
Texas	21	24	23		162	189	80	642	228	228	5	12
MOUNTAIN												
Montana	0	0	1		1			18	121	72	1	0
Idaho	0	0	0			5		5	74	35	2	6
Wyoming	0	0	1			7	2	25	49	18	2	0
Colorado	5	5	7		12	27	12	50	64	69	2	4
New Mexico	3	2	1		5	1		17	6	11	1	0
Arizona	0	2	2		26	44	31	35	20	34	0	0
Utah	0	0	0		1	12	1	43	98	98	0	3
Nevada	4	0	0					9	1	0	0	0
PACIFIC												
Washington	6	5	0		1			123	130	141	4	6
Oregon	4	2	1		6	2	3	54	59	80	0	3
California	19	19	15		9	77	56	1,710	693	693	11	24
Total	168	100	196	420	609	451	7,556	14,022	8,695	219	335	45
25 weeks	5,396	5,983	6,394	334,931	76,886	149,068	570,515	499,064	444,331	11,623	11,766	1,175

See footnotes at end of table.

Telegraphic morbidity reports from State health officers for the week ended June 24, 1944, and comparison with corresponding week of 1943 and 5-year median—Con.

Division and State	Pollomyelitis			Scarlet fever			Smallpox			Typhoid and paratyphoid fever ¹		
	Week ended—		Me-dian 1939-43	Week ended—		Me-dian 1939-43	Week ended—		Me-dian 1939-43	Week ended—		Me-dian 1939-43
	June 24, 1944	June 26, 1943		June 24, 1944	June 26, 1943		June 24, 1944	June 26, 1943		June 24, 1944	June 26, 1943	
NEW ENGLAND												
Maine.....	0	0	0	16	14	7	0	0	0	1	0	0
New Hampshire.....	0	0	0	1	2	2	0	0	0	0	0	0
Vermont.....	0	0	0	6	6	2	0	0	0	0	0	0
Massachusetts.....	0	0	0	164	256	135	0	0	0	3	3	3
Rhode Island.....	0	0	0	7	25	5	0	0	0	0	0	1
Connecticut.....	1	3	0	25	44	20	0	0	0	1	1	1
MIDDLE ATLANTIC												
New York.....	9	6	1	219	189	217	0	0	0	2	7	10
New Jersey.....	1	1	0	71	35	70	0	0	0	1	1	2
Pennsylvania.....	2	1	1	141	85	163	0	0	0	2	4	7
EAST NORTH CENTRAL												
Ohio.....	7	1	1	97	62	101	0	0	0	3	7	5
Indiana.....	1	0	0	20	18	23	0	0	0	0	1	4
Illinois.....	5	0	2	75	87	156	0	0	5	4	4	4
Michigan ²	2	0	2	158	52	148	0	0	0	1	12	3
Wisconsin.....	0	0	0	104	136	67	3	0	0	1	1	1
WEST NORTH CENTRAL												
Minnesota.....	4	0	1	52	19	20	0	0	0	0	0	0
Iowa.....	0	0	0	27	15	15	0	0	2	0	0	2
Missouri.....	0	1	1	22	14	14	0	0	0	2	0	1
North Dakota.....	0	0	0	10	5	6	0	0	0	0	0	0
South Dakota.....	0	0	0	6	3	4	0	0	0	0	0	0
Nebraska.....	0	0	0	13	5	9	0	0	0	0	0	0
Kansas.....	1	0	0	9	21	18	0	1	0	3	0	1
SOUTH ATLANTIC												
Delaware.....	0	0	0	2	1	4	0	0	0	0	1	1
Maryland ²	0	0	0	58	27	22	0	0	0	2	2	1
District of Columbia.....	0	0	0	17	2	6	0	0	0	0	0	0
Virginia.....	4	1	1	23	10	7	0	0	0	2	5	5
West Virginia.....	0	0	0	26	11	13	0	0	0	2	4	4
North Carolina.....	42	0	1	11	11	11	0	0	0	6	1	5
South Carolina.....	2	1	1	12	1	1	0	0	0	4	1	4
Georgia.....	1	0	1	7	7	6	0	0	0	5	13	15
Florida.....	1	0	1	6	1	1	0	0	0	2	2	2
EAST SOUTH CENTRAL												
Kentucky.....	17	1	1	15	11	17	0	4	3	3	3	7
Tennessee.....	0	1	1	19	7	14	0	0	0	2	0	6
Alabama.....	3	2	2	3	3	4	0	0	0	2	2	3
Mississippi ²	2	0	0	5	3	3	0	0	0	6	5	3
WEST SOUTH CENTRAL												
Arkansas.....	2	2	2	0	1	2	0	0	1	2	3	6
Louisiana.....	7	2	1	4	1	5	0	0	0	6	9	11
Oklahoma.....	2	8	1	3	9	7	0	0	0	3	2	5
Texas.....	4	39	3	23	28	18	1	0	0	16	18	21
MOUNTAIN												
Montana.....	0	0	0	13	2	6	0	0	0	1	0	1
Idaho.....	0	0	0	6	60	4	0	0	0	0	0	1
Wyoming.....	0	0	0	4	6	3	0	0	0	0	0	0
Colorado.....	2	0	0	31	24	17	0	0	2	2	3	3
New Mexico.....	0	2	0	7	2	4	0	0	0	1	0	1
Arizona.....	0	6	3	12	13	3	0	0	0	4	0	1
Utah ²	1	0	0	16	13	5	0	0	0	0	0	0
Nevada.....	0	0	0	0	0	0	0	0	0	0	0	0
PACIFIC												
Washington.....	0	0	0	71	23	19	0	1	0	0	2	2
Oregon.....	0	0	0	35	10	7	0	2	2	1	1	1
California.....	3	58	14	164	129	107	0	0	1	8	6	6
Total.....	126	136	69	1,836	1,500	1,578	4	8	19	104	124	155
25 weeks.....	822	894	607	139,920	91,042	91,042	263	568	1,081	2,004	1,666	2,598

See footnotes at end of table.

June 30, 1944

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Telegraphic morbidity reports from State health officers for the week ended June 24, 1944, and comparison with corresponding week of 1943 and 5-year median

Division and State	Whooping cough			Week ended June 24, 1944									
	Week ended		Median 1939- 43	An- thrax	Dysentery			En- ceph- alitis, infectious	Lep- rosy	Rocky Mt. spot- ted fever	Tula- remia	Ty- phus fever	
	June 24, 1944	June 26, 1943			Ame- bic	Bacil- lary	Un- spec- ified						
NEW ENGLAND													
Maine.....	14	6	22	0	0	0	0	0	0	0	0	0	
New Hampshire.....	0	4	4	0	0	0	0	0	0	0	0	0	
Vermont.....	18	12	21	0	0	0	0	0	0	0	0	0	
Massachusetts.....	58	96	144	0	0	0	0	0	0	0	0	0	
Rhode Island.....	3	34	20	0	0	0	0	0	0	0	0	0	
Connecticut.....	24	21	54	0	0	0	0	0	0	0	0	0	
MIDDLE ATLANTIC													
New York.....	110	245	300	0	0	7	0	2	0	3	0	2	
New Jersey.....	46	181	181	1	1	0	0	0	0	1	0	0	
Pennsylvania.....	80	277	277	2	1	0	0	0	0	1	0	0	
EAST NORTH CENTRAL													
Ohio.....	83	168	173	0	0	0	0	1	0	0	0	0	
Indiana.....	12	60	43	0	0	0	0	0	0	0	0	0	
Illinois.....	71	156	156	0	1	0	0	1	0	0	0	0	
Michigan ²	64	249	173	0	0	4	0	0	0	0	0	0	
Wisconsin.....	62	225	169	0	0	0	0	0	0	0	0	0	
WEST NORTH CENTRAL													
Minnesota.....	25	48	39	0	5	0	0	0	0	0	0	0	
Iowa.....	8	59	28	0	0	0	0	0	0	0	0	0	
Missouri.....	20	36	28	0	0	0	1	0	0	0	0	0	
North Dakota.....	13	7	13	0	0	0	0	0	0	0	0	0	
South Dakota.....	19	5	1	0	0	0	0	0	0	0	0	0	
Nebraska.....	34	11	11	0	0	0	0	0	0	0	0	0	
Kansas.....	29	88	56	0	0	0	0	0	0	0	0	0	
SOUTH ATLANTIC													
Delaware.....	0	12	7	0	0	0	0	0	0	0	0	0	
Maryland ²	90	148	75	0	0	0	1	0	0	9	0	1	
District of Columbia.....	1	38	28	0	0	0	0	0	0	0	0	0	
Virginia.....	111	190	103	0	0	0	250	0	0	4	1	0	
West Virginia.....	7	86	33	0	0	0	0	0	0	0	0	0	
North Carolina.....	184	273	155	0	0	0	0	0	0	6	2	3	
South Carolina.....	41	70	70	0	0	37	0	0	0	0	0	1	
Georgia.....	13	37	37	0	1	12	67	0	0	1	0	20	
Florida.....	10	23	13	0	2	3	0	0	0	0	0	6	
EAST SOUTH CENTRAL													
Kentucky.....	103	80	52	0	0	12	0	0	0	3	0	0	
Tennessee.....	34	62	62	0	0	0	6	0	0	0	0	0	
Alabama.....	48	96	40	0	0	0	0	1	0	0	0	16	
Mississippi ²				0	0	0	0	0	0	0	1	7	
WEST SOUTH CENTRAL													
Arkansas.....	16	31	31	0	0	10	0	0	0	0	5	0	
Louisiana.....	1	13	16	0	0	6	0	0	0	0	0	4	
Oklahoma.....	1	68	16	0	0	0	0	0	0	0	0	0	
Texas.....	215	566	359	0	38	443	0	2	0	0	1	37	
MOUNTAIN													
Montana.....	9	54	15	0	0	0	0	0	0	0	1	0	
Idaho.....	9	1	7	0	0	0	0	0	0	0	0	0	
Wyoming.....	15	0	3	0	0	0	0	0	0	3	1	0	
Colorado.....	21	42	42	0	0	0	0	0	0	2	0	0	
New Mexico.....	4	13	17	0	0	0	1	0	0	0	0	0	
Arizona.....	13	27	27	0	0	0	45	0	0	0	0	0	
Utah ²	76	67	67	0	0	0	0	0	0	1	0	0	
Nevada.....	0	0	0	0	0	0	0	0	0	0	0	0	
PACIFIC													
Washington.....	10	53	53	0	0	0	0	0	0	6	0	0	
Oregon.....	9	49	30	0	0	0	0	0	0	0	0	0	
California.....	82	282	282	0	5	14	0	2	0	0	0	0	
Total.....	1,916	4,369	3,862	3	54	548	371	9	0	34	12	97	
25 weeks.....	45,334	101,909	98,028	22	676	8,033	2,493	274	15	144	280	1,295	
25 weeks, 1943.....				34	917	5,718	1,552	274	13	160	465	1,204	

¹ New York City only.² Period ended earlier than Saturday.

³ Including paratyphoid fever cases reported separately as follows: Massachusetts 3, Illinois 1, Michigan 1, Georgia 2, Kentucky 1, Texas 1, California 1.

WEEKLY REPORTS FROM CITIES

City reports for week ended June 10, 1944

This table lists the reports from 89 cities of more than 10,000 population distributed throughout the United States, and represents a cross section of the current urban incidence of the diseases included in the table.

	Diphtheria cases	Encephalitis, infectious, cases	Influenza		Measles cases	Meningitis, meningococcus, cases	Pneumonia deaths	Poliomyelitis cases	Scarlet fever cases	Smallpox cases	Typhoid and paratyphoid fever cases	Whooping cough cases
			Cases	Deaths								
NEW ENGLAND												
Maine:												
Portland	0	0		0	50	0	2	0	11	0	0	1
New Hampshire:												
Concord	0	0		0	8	0	0	0	0	0	0	0
Vermont:												
Barre	0	0		0	0	0	0	0	0	0	0	0
Massachusetts:												
Boston	2	1		0	123	9	8	0	49	0	0	2
Fall River	0	0		0	22	0	1	0	1	0	0	0
Springfield	0	0		0	24	1	0	0	12	0	0	8
Worcester	0	0		0	7	0	2	0	26	0	1	7
Rhode Island:												
Providence	0	0		0	5	2	1	0	5	0	0	11
Connecticut:												
Bridgeport	0	0		0	4	1	0	0	0	0	0	1
Hartford	1	0		0	9	0	2	0	12	0	0	5
New Haven	0	0		0	15	0	1	0	0	0	0	0
MIDDLE ATLANTIC												
New York:												
Buffalo	0	0		1	9	2	2	0	6	0	0	0
New York	6	1		0	350	20	58	3	163	0	5	43
Rochester	0	0		0	45	0	2	0	7	0	1	7
Syracuse	0	0		0	2	2	0	0	1	0	0	3
New Jersey:												
Camden	0	0		0	3	0	1	0	6	0	0	0
Newark	0	0	1	0	103	1	3	0	23	0	0	5
Trenton	0	0		0	0	1	5	0	2	0	0	3
Pennsylvania:												
Philadelphia	1	0	4	0	44	11	12	0	55	0	1	6
Pittsburgh	0	0	2	2	4	3	7	2	11	0	0	3
Reading	0	0		0	0	2	2	0	1	0	0	1
EAST NORTH CENTRAL												
Ohio:												
Cincinnati	1	0		1	33	6	3	0	11	0	0	5
Cleveland	0	0		0	19	3	5	1	48	0	0	7
Columbus	0	0	1	1	8	1	2	0	4	0	0	3
Indiana:												
Fort Wayne	0	0		0	0	0	0	0	1	0	0	0
Indianapolis	2	0		0	21	2	3	0	32	0	0	12
South Bend	0	0		0	3	0	0	0	0	0	0	2
Terre Haute	0	0		0	4	0	0	0	0	0	0	3
Illinois:												
Chicago	3	0		0	133	9	20	0	52	0	1	13
Michigan:												
Detroit	9	0		0	117	7	5	1	67	0	1	31
Flint	0	0		0	3	0	2	0	10	0	0	9
Grand Rapids	0	0		0	2	0	0	0	4	0	0	2
Wisconsin:												
Kenosha	0	0		0	124	0	0	0	0	0	0	5
Milwaukee	0	0		0	219	4	2	0	24	0	0	17
Racine	0	0		0	166	0	2	0	0	0	0	8
Superior	0	0		0	6	0	0	0	9	0	0	0
WEST NORTH CENTRAL												
Minnesota:												
Duluth	0	0		0	180	0	0	0	7	0	0	1
Minneapolis	0	0		0	79	2	0	0	15	0	1	6
St. Paul	0	0		0	33	3	10	0	25	0	0	2
Missouri:												
Kansas City	0	0		1	18	3	4	0	7	0	0	0
St. Joseph	0	0		0	0	0	0	0	3	0	0	0
St. Louis	0	0		0	9	5	4	0	13	0	0	3
North Dakota:												
Fargo	0	0		0	1	0	0	0	0	0	0	9

City reports for week ended June 10, 1944—Continued

	Diphtheria cases	Encephalitis, infectious, cases	Influenza		Measles cases	Meningitis, meningococci, cases	Pneumonia deaths	Polymyelitis cases	Scarlet fever cases	Smallpox cases	Typhoid and paratyphoid fever cases	Whooping cough cases
			Cases	Deaths								
WEST NORTH CENTRAL— continued												
Nebraska:												
Omaha.....	1	0	—	0	8	0	3	0	4	0	0	0
Kansas:												
Topeka.....	0	0	—	0	13	0	0	0	5	0	0	0
Wichita.....	0	0	—	0	5	0	2	0	1	0	0	0
SOUTH ATLANTIC												
Delaware:												
Wilmington.....	0	0	—	0	0	0	1	0	1	0	0	0
Maryland:												
Baltimore.....	5	0	—	0	76	2	9	0	33	0	0	38
Cumberland.....	0	0	—	0	0	0	0	0	0	0	0	0
Frederick.....	0	0	—	0	0	0	0	0	0	0	0	0
District of Columbia:												
Washington.....	1	0	—	0	60	2	7	0	32	0	1	1
Virginia:												
Lynchburg.....	0	0	—	0	1	0	1	0	6	0	0	0
Richmond.....	0	0	—	0	1	0	1	0	1	0	0	3
Roanoke.....	0	0	—	0	3	0	0	0	1	0	0	2
West Virginia:												
Charleston.....	0	0	—	0	0	0	0	0	2	0	0	0
Wheeling.....	0	0	—	0	47	0	0	0	0	0	0	1
North Carolina:												
Raleigh.....	0	0	—	0	18	0	0	0	0	0	0	0
Wilmington.....	0	0	—	0	11	0	2	0	0	0	0	10
Winston-Salem.....	1	0	—	0	4	0	0	0	0	0	0	0
South Carolina:												
Charleston.....	0	0	—	0	0	0	0	0	0	0	0	3
Georgia:												
Atlanta.....	1	0	1	1	9	0	2	0	1	0	0	0
Brunswick.....	0	0	—	0	1	0	2	0	0	0	0	0
Savannah.....	0	0	1	1	0	0	1	0	0	0	0	0
Florida:												
Tampa.....	0	0	2	0	10	1	1	0	0	0	1	4
EAST SOUTH CENTRAL												
Tennessee:												
Memphis.....	0	0	—	0	9	1	1	1	3	0	1	3
Nashville.....	0	0	—	0	13	0	2	0	1	0	0	2
Alabama:												
Birmingham.....	0	0	—	1	3	0	3	0	2	0	0	0
Mobile.....	0	0	—	1	0	0	2	0	1	0	0	0
WEST SOUTH CENTRAL												
Arkansas:												
Little Rock.....	0	0	—	0	2	0	0	0	0	0	0	0
Louisiana:												
New Orleans.....	1	0	4	1	6	2	5	3	0	0	0	2
Shreveport.....	0	0	—	0	2	0	5	0	0	0	0	0
Texas:												
Dallas.....	1	0	1	1	11	0	0	0	3	0	0	8
Galveston.....	0	0	—	0	0	0	2	0	2	0	0	0
Houston.....	0	0	—	0	1	0	4	0	3	0	1	3
San Antonio.....	0	0	1	1	0	0	2	0	0	0	0	1
MOUNTAIN												
Montana:												
Billings.....	0	0	—	0	8	0	0	0	2	0	0	1
Great Falls.....	0	0	—	0	1	0	2	0	4	0	0	1
Helena.....	0	0	—	0	1	0	0	0	0	0	0	0
Missoula.....	0	0	—	0	13	0	2	0	1	0	0	0
Idaho:												
Boise.....	0	0	—	0	0	0	0	0	0	0	0	0
Colorado:												
Denver.....	4	0	—	0	29	1	7	0	13	0	0	5
Pueblo.....	0	0	—	0	0	0	1	0	0	0	0	0
Utah:												
Salt Lake City.....	0	0	—	0	25	1	0	0	26	0	0	8

City reports for week ended June 10, 1944—Continued

	Diphtheria cases	Encephalitis, infectious, cases	Influenza		Measles cases	Meningitis, meningococcus, cases	Pneumonia deaths	Polliomyelitis cases	Scarlet fever cases	Smallpox cases	Typhoid and paratyphoid fever cases	Whooping cough cases
			Cases	Deaths								
PACIFIC												
Washington:												
Seattle.....	0	0	-----	0	56	0	4	0	18	0	0	1
Spokane.....	0	0	-----	0	19	0	2	0	15	0	0	0
Tacoma.....	0	0	-----	0	28	1	1	0	11	0	0	1
California:												
Los Angeles.....	6	0	3	0	265	6	2	0	35	0	0	5
Sacramento.....	0	0	-----	0	34	0	2	0	9	0	0	3
San Francisco.....	1	0	1	1	224	5	10	0	45	0	0	5
Total.....	47	2	22	14	3,024	122	262	11	1,004	0	15	346
Corresponding week, 1943.....	53	-----	52	16	6,316	-----	340	-----	864	1	17	1,304
Average, 1939-43.....	64	-----	45	14	4,360	-----	281	-----	963	4	24	1,194

¹ 3-year average, 1941-43.² 5-year median.*Dysentery, amebic.*—Cases: Tampa 1.*Dysentery, bacillary.*—Cases: St. Louis, 2; Charleston, S. C., 8; Nashville, 1; Los Angeles, 12.*Dysentery, unspecified.*—Cases: Shreveport, 1; San Antonio, 17.*Leprosy.*—Cases: San Francisco, 1.*Rocky Mountain spotted fever.*—Cases: Richmond, 1.*Typhus fever, endemic.*—Cases: Tampa, 1; Mobile, 1; Houston, 1.

Rates (annual basis) per 100,000 population, by geographic groups, for the 89 cities in the preceding table (estimated population, 1943, 34,322,300)

	Diphtheria case rates	Encephalitis, infectious, case rates	Influenza		Measles case rates	Meningitis, meningococcus, case rates	Pneumonia death rates	Polliomyelitis case rates	Scarlet fever case rates	Smallpox case rates	Typhoid and paratyphoid fever case rates	Whooping cough case rates
			Case rates	Death rates								
New England.....	7.8	2.6	0.0	0.0	698	34.0	44.4	0.0	303	0.0	2.6	91
Middle Atlantic.....	3.2	0.5	3.2	1.4	259	19.4	42.6	2.3	127	0.0	3.2	33
East North Central.....	9.2	0.0	0.6	1.2	526	19.6	27.0	1.2	161	0.0	1.2	72
West North Central.....	2.0	0.0	0.0	2.0	688	25.9	45.8	0.0	159	0.0	2.0	24
South Atlantic.....	13.1	0.0	6.5	3.3	397	8.2	44.1	0.0	126	0.0	3.3	101
East South Central.....	0.0	0.0	0.0	11.8	148	5.9	47.2	5.9	41	0.0	5.9	30
West South Central.....	5.7	0.0	17.2	8.6	63	5.7	51.7	8.6	23	0.0	2.9	40
Mountain.....	31.8	0.0	0.0	0.0	612	15.9	95.3	0.0	365	0.0	0.0	119
Pacific.....	11.1	0.0	6.3	1.6	990	19.0	33.2	0.0	210	9.0	0.0	24
Total.....	7.2	0.3	3.4	2.1	461	18.6	39.9	1.7	153	0.0	2.3	53

FOREIGN REPORTS

CANADA

Provinces—Communicable diseases—Week ended May 27, 1944.—During the week ended May 27, 1944, cases of certain communicable diseases were reported by the Dominion Bureau of Statistics of Canada as follows:

Disease	Prince Edward Island	Nova Scotia	New Brunswick	Quebec	Ontario	Manitoba	Saskatchewan	Alberta	British Columbia	Total
Chickenpox		38		153	266	30	19	71	151	728
Diphtheria		8	6	20	1	6				42
Dysentery (bacillary)				14					1	15
German measles		18		162	77	9	34	14	50	364
Influenza					10				2	12
Measles	2	56	4	732	685	237	82	94	38	1,930
Meningitis, meningococcus					2					2
Mumps		9		196	150	27	9	72	25	488
Polio-myelitis					1					2
Scarlet fever		18	12	87	105	51	8	77	74	522
Tuberculosis (all forms)	1	5	5	174	36	18	17		64	320
Typhoid and paratyphoid fever			2	5	1			2	1	11
Undulant fever					1	1				2
Whooping cough		31		41	38	1	5	11	39	166

JAMAICA

Notifiable diseases—4 weeks ended June 3, 1944.—During the 4 weeks ended June 3, 1944, cases of certain notifiable diseases were reported in Kingston, Jamaica, and in the island outside of Kingston, as follows:

Disease	Kingston	Other localities	Disease	Kingston	Other localities
Chickenpox	20	60	Leprosy		5
Diphtheria	2	2	Tuberculosis	37	62
Dysentery	2	1	Typhoid fever	13	43
Erysipelas	1	2	Typhus fever	13	3

SWEDEN

Notifiable diseases—March 1944.—During the month of March 1944, cases of certain notifiable diseases were reported in Sweden as follows:

Disease	Cases	Disease	Cases
Cerebrospinal meningitis	8	Polio-myelitis	35
Diphtheria	281	Scarlet fever	3,071
Carriers	222	Syphilis	90
Dysentery	111	Typhoid fever	2
Gonorrhea	1,567	Undulant fever	3
Hepatitis, epidemic	694	Weil's disease	13
Paratyphoid fever	14		

WORLD DISTRIBUTION OF CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER

From medical officers of the Public Health Service, American consuls, International Office of Public Health, Pan American Sanitary Bureau, health section of the League of Nations, and other sources. The reports contained in the following tables must not be considered as complete or final as regards either the list of countries included or the figures for the particular countries for which reports are given.

CHOLERA

[C indicates cases]

NOTE.—Since many of the figures in the following tables are from weekly reports, the accumulated totals are for approximate dates.

Place	January- March 1944	April 1944	May 1944—week ended—			
			6	13	20	27
ASIA						
Ceylon.....	C	2				
India.....	C	42,788	10,599			
Calcutta.....	C	725	681	274	138	155
Chittagong.....	C	63				
Madras.....	C	36				
Negapatam.....	C	17				

PLAGUE

[C indicates cases; D, deaths; P, present]

AFRICA							
Belgian Congo.....	C	3					
Plague-infected rats.....	P						
British East Africa:							
Kenya.....	C	1					
Uganda.....	C	3	1				
Egypt.....	C	134	117	44	44	51	40
Port Said.....	C	1	6	1	2		3
Suez.....	C	117	23	3	3	2	3
French West Africa: Dakar.....	C		7	1	1		
Madagascar.....	C	50					
Morocco (French).....	C	20	2				
Rhodesia, northern.....	C	1					
Union of South Africa.....	C	23					
ASIA							
China: Foochow.....	C	P					
India.....	C	4,811	1,621				
Indochina.....	C	17	13		6		
Palestine.....	C	1					
SOUTH AMERICA							
Bolivia: Chuquisaca Department.....	C	4					
Ecuador: Chimborazo Department.....	C	1					
Peru:							
Libertad Department.....	C	5					
Lima Department.....	C	16					
OCEANIA							
Hawaii Territory:							
Hamakua District.....	D	14					
Plague-infected rats.....		33	8				

¹ Includes 1 death from pneumonic plague.

² 53 fleas were also proved positive for plague on March 7, 1944.

³ Includes 11 plague-infected mice.

⁴ Includes 1 plague-infected mouse.

SMALLPOX

[C indicates cases; P, present]

Place		January-March 1944	April 1944	May 1944—week ended—			
				6	13	20	27
AFRICA							
Algeria.....	C	364	90				1 141
Angola.....	C	20					
Basutoland.....	C	31					
Bechuanaland.....	C	7					
Belgian Congo.....	C	747	171	8	29		
British East Africa:							
Kenya.....	C	1,834	358	69	82	35	
Mombasa.....	C	95	31	1	3	1	4
Tanganyika.....	C	427	306	29	43	48	
Uganda.....	C	900	613	150	88	123	
Cameroon (French).....	C	190	143				
Dahomey.....	C	20	24				
Egypt.....	C	4,768	1,236			382	
French Equatorial Africa.....	C	418					
French Guinea.....	C	198	171				
French West Africa: Dakar.....	C		4				
Gambia.....	C	13					
Gold Coast.....	C	5					
Ivory Coast.....	C	255	84				
Morocco (French).....	C	522	54				
Mozambique.....	C	1					
Nigeria.....	C	1,648	443	174	99	89	
Niger Territory.....	C	391	65				
Senegal.....	C	59	26				
Sudan (French).....	C	1,167	533				
Tunisia.....	C	5					
Union of South Africa.....	C	29	1	4	1		
ASIA							
Arabia.....	C	2 7	2				
Ceylon.....	C	7	1				
China: Kunming ³	C	7	18	7	1	1	6
India.....	C	102,820	43,302				
Indochina.....	C	990	275				
Iran.....	C	1					
Iraq.....	C	22	1				
Palestine.....	C	4	51	10		10	10
Syria and Lebanon.....	C	122	43		2		
EUROPE							
Gibraltar.....	C	P					
Great Britain:							
Birkenhead.....	C				1		
London.....	C	4 12					
Greece: Evros Department.....	C	209					
Portugal.....	C	9	4			1	
Spain.....	C	42	72		5		
Turkey.....	C	5,016					
NORTH AMERICA							
Guatemala.....	C		1				
Honduras.....	C	6					
Mexico.....	C	908					
SOUTH AMERICA							
Bolivia.....	C	85	77				
Brazil.....	C	6	19		7	10	11
Colombia.....	C	110	38				
Ecuador.....	C	4					
Peru.....	C	47					
Lima.....	C	19					
Venezuela.....	C	48	29				

¹ For the month of May 1944.² Includes 4 imported cases.³ Yunnan Fu.⁴ Includes 1 case imported from the Middle East.

TYPHUS FEVER

[C indicates cases]

Place		January-March 1944	April 1944	May 1944—week ended—			
				6	13	20	27
AFRICA							
Algeria.....	C	303	188				¹ 234
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¹ For the month of May 1944.² For the period May 1-20, 1944.³ A report dated Mar. 30, 1944, states that an estimated 800 deaths from typhus fever have occurred.⁴ Yunnan Fu.⁵ For 3 weeks.⁶ Cases of typhus fever listed in this area are probably of endemic type.

YELLOW FEVER

[C indicates cases; D, deaths]

Place	January- March 1944	April 1944	May 1944—week ended—			
			6	13	20	27
AFRICA						
Belgian Congo:						
Babeyru.....	D	1				
Bondo. ¹						
Leopoldville.....	C	1				
Gold Coast: Tamale.....	C	1				
EUROPE						
Portugal: Lisbon. ²						
SOUTH AMERICA						
Brazil:						
Acre Territory.....	D	1				
Matto Grosso State.....	D	3				
Colombia:						
Boyaca Department.....	D	1				
Caldas Department.....	D	1				
Santander Department.....	D	2				

¹ For the week ended June 3, 1944, 1 death from yellow fever was reported in Bondo, Stanleyville Province, Belgian Congo.

² Suspected.

³ According to information dated Jan. 21, 1944, it is reported that a vessel which called at the islands of Sao Tome and Cape Verde arrived at Lisbon, Portugal, with cases of yellow fever on board.

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UNITED STATES PUBLIC HEALTH SERVICE

THOMAS PARRAN, *Surgeon General*

DIVISION OF PUBLIC HEALTH METHODS

G. ST. J. PERROTT, *Chief of Division*

The PUBLIC HEALTH REPORTS, first published in 1878 under authority of an act of Congress of April 29 of that year, is issued weekly by the United States Public Health Service through the Division of Public Health Methods, pursuant to the following authority of law: United States Code, title 42, sections 7, 30, 93; title 44, section 220.

It contains (1) current information regarding the prevalence and geographic distribution of communicable diseases in the United States, insofar as data are obtainable, and of cholera, plague, smallpox, typhus fever, yellow fever, and other important communicable diseases throughout the world; (2) articles relating to the cause, prevention, and control of disease; (3) other pertinent information regarding sanitation and the conservation of the public health.

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